Amendments to the Specification:

Please replace paragraph [0020] with the following amended paragraph:

[0020] ~~FIG.~~ Figures 4A to 4C are ~~is~~ a series of graphs depicting anti-PlpE (A), anti-*M. haemolytica* leukotoxin (B), and anti-*M. haemolytica* whole cells (C) in cattle vaccinated with PRESPONSE, PRESPONSE plus 100 µg of rPlpE, or nonvaccinated.

Please replace paragraph [0029] with the following amended paragraph:

[0029] The expression of rPlpE was done according to the protocol recommended by the manufacturer of the vector and the expression host (Invitrogen, CA). Briefly, single colonies of BL21(DE3)pLysS harboring the truncated *plpE* in pRSETA, were inoculated into appropriate volumes of LB broth with 50 µg/ml ampicillin and 34 µg/ml chloramphenicol. The culture were incubated at 37°C until A_{600} = 0.5 was attained at which time the synthesis of the recombinant protein was induced by adding IPTG (1mM final concentration) and the induction was continued for at least 3 hours. In order to purify rPlpE, the culture was harvested and lysed by sonication. The cellular debris was then removed by centrifugation and the recombinant protein was loaded onto an affinity column packed by ~~ProBond~~ PROBOND nickel-chelating resin that selectively binds recombinant proteins with 6 histidine residues (His-Tag) at either the N- or Carboxy-terminus. In this instance, the His-Tag is at the N-terminus. The recombinant protein bound to the resin was then eluted with either a low pH buffer or competition with imidazole. The purity

of each preparation was determined by SDS-PAGE followed Coomassie stain and Western blot with murine anti-PlpE ascites fluid.

Please replace paragraph **[0053]** with the following amended paragraph:

[0053] Anti-rPlpE antibodies against specific regions of PlpE were purified using the affinity columns described above. The ~~Econo-Column~~ ECONO-COLUMN with NHS-activated Sepharose coupled to an rPlpE of interest was fitted with a Flow adaptor according to the recommendation of the manufacturer (BioRad, Hercules, CA). The affinity column was equilibrated by applying Dulbecco's Phosphate Buffered Saline (DPBS) at a flow rate of 1ml/min. Hyperimmune serum produced by immunizing calves with the intact rPlpE was mixed with DPBS in a ratio of 1 to 10 and passed through Nalgene 0.45μm PES filters (Nalge, Rochester, NY). The filtered serum was then applied to the equilibrated column via peristaltic pump at a flow rate of 1 ml/min. The flow thru was re-applied to the column several times to re-extract the serum by connecting the flow through to the reservoir of the initial serum. The column was then washed with DPBS. The complete removal of nonspecific proteins was determined with the help of the UV monitor attached to a chart recorder. Once there was no indication of nonspecific protein in the flow through, the specifically bound antibody was eluted with 100 mM Glycine Buffer (100 mM Glycine, 140 mM NaCl, pH 3.0) by collecting fractions in microfuge tubes containing 1/10 vol of 1 M Tris-HCl, pH 8.0. The absorbance of each fraction was determined at 280nm. Those fractions that had a reading at least 2 – 3 times the background

were pooled and dialyzed overnight against DPBS at 4°C in a ~~Slide-A-Lyzer~~ SLIDE-A-LYZER

Dialysis Cassette (Pierce, Rockford, IL). The concentration of affinity purified antibody was

determined with BCA Protein Assay Kit (Pierce[[.]], Rockford, IL). More specific antibodies

against rPlpE with 28, 76 and 150 amino acids deletions on their N-termini, rPlpEΔN28

(pSAC63), rPlpEΔN76 (pSAC64) and rPlpEΔN150 (pSAC65), respectively, were purified as

described.

Please replace paragraph **[0056]** with the following amended paragraph:

[0056] The peptide array was probed with anti-PlpE hyperimmune sera as follows. Prior to

blotting, membranes with the custom spots were blocked with ~~SuperBlock~~ SUPERBLOCK Dry

Blend (Pierce, Rockford, IL) blocking buffer in TBS, pH 7.4. The membrane was then incubated

in blocking buffer containing a primary antibody at a dilution of 1: 1000 to 1: 5000 for an hour.

Following several washes with TBS, pH 7.4, supplemented with 0.05% ~~Tween-20~~ TWEEN -20,

0.2% ~~Triton-X-100~~ TRITON-X-100 (TBSTT), the membrane was incubated in ~~Superblock~~

SUPERBLOCK containing a goat anti-bovine or anti-mouse secondary antibody conjugated to

Horse Radish Peroxidase (KPL, Gaithsburg, MD) at dilutions 1:100,000 to 1:200,000 for one

hour. The membrane was washed several times with TBSTT. The peptide array was incubated

with SuperSignal West Pico Chemiluminescent Substrate working solution (0.125 ml/cm2) for 5

minutes, placed in plastic membrane protector and exposed to a CL-X Posure (Pierce, Rockford,

IL) X-Ray film for varying durations of time. The X-Ray film was then developed in a ~~Konica~~

KONICA SRX-101A Medical Film Processor (Taiwan). The developed X-Ray film was scanned

by ~~Arcus~~ ARCUS 1200 ~~Agfa~~ AGFA scanner (Taiwan), and scanned images were analyzed using

~~Gene Pix~~ GENEPIX Pro 4.0 (Axon Instruments, Union City, CA). Signal intensities were

defined as median pixel intensity following subtraction of local median background signal. The

peptide array was stripped with RESTORE™ Western Blot Stripping Buffer (Pierce, Rockford,

IL) according to the procedure recommended by the manufacturer before it was probed with a

different anti-PlpE antibody. This was repeated several times with anti-PlpE antibodies obtained

from different sources or purified in varieties of ways.

Please replace paragraph **[0058]** with the following amended paragraph:

[0058] Putative antigenic regions in PlpE were identified by using the ~~MacVector~~

MACVECTOR 7.0 software that employed algorithms such as antigenic index, hydrophilicity

and surface probability. However, the identification of epitopes was done with a peptide array

comprising 109 overlapping 13-mer peptides that were synthesized by the chemistry described

earlier. The peptides were covalently bound to derivatized cellulose membrane by the C-

terminus and have a free N-terminus. Anti-PlpE hyperimmune antibodies purified by any

number of the methods described earlier were used to probe the peptide array. The custom spots

were stripped and probed several times. When bovine antibody against surface exposed

components of PlpE that was affinity purified with intact *M. haemolytica* cells was used to probe

the peptide array a total of 8 distinct regions (E1 – 8) were identified. (FIG. 6.)   Epitope 1

(PNHPKPVLVPKTQNNL) (SEQ ID NO: 11) spans 3 peptides; epitope 2

(QNASQAQNAPQAQNAPQAQNAPQVENAPQAQNAPQVENAPQAE) (SEQ ID NO: 12),

11 peptides;   epitope 3 (GSFDKIGSVKLNK) (SEQ ID NO: 13), 3 peptides; epitope 4

(KLGTPPKFDKVSGKKIIEE) (SEQ ID NO: 14), 6 peptides; epitope 5 (LIRRSDDLFYGYY)

(SEQ ID NO: 15), 3 peptides; epitope 6 (ADKFSQYFVVYDE) (SEQ ID NO: 16), 3 peptides;

epitope 7 (NISDKLTATYRKK) (SEQ ID NO: 17), 2 peptides; and epitope 8

(PHTKEFAARISKL) (SEQ ID NO: 18). More or less the same set of epitopes, albeit with

decreasing intensities, were picked up when whole serum obtained from cows with a naturally

high anti-PlpE antibody titer that were also challenged with live *M. haemolytica* was used. The

signal intensities of all of the epitopes with the exception of epitope 2 were much lesser in this

blot than in the earlier. The purification of IgG from the latter serum with Protein G affinity

columns did not alter the above result in that exactly the same putative epitopes were identified

suggesting that IgG was the class of immunoglobulins involved in this immune response. When

whole hyperimmune serum from calves immunized with rPlpE was used to probe the stripped

peptide array exactly the same set of peptides mentioned above were identified once again

confirming the binding capacity of the above indicated stretches of amino acids along PlpE. On

the other hand, when sera from calves that were given live *M. haemolytica* were used to probe

the peptide array, epitope 2 was the only one that was picked up. According to the manufacturers

of the custom spots, non-specific binding of the antibody-enzyme conjugate may occur to

peptides that contain combinations of basic amino acids. When goat anti-bovine-HRP, the

secondary antibody used in this project, was used to probe the spots, epitopes 1, 3, 4, 7, and 8

were picked up. The same sets of epitopes were identified when the array was probed with rabbit

anti-bovine-HRP, showing putative epitopes 1, 3, 4, 7, and 8 were not true epitopes. In order to

identify spots that would non-specifically bind bovine immunoglobulins, serum from colostrum

deprived new born calf was used to probe the array. Interestingly, in addition to the putative

epitopes identified by the secondary antibody-enzyme conjugates, i.e., 1, 3, 4, 7, and 8, epitopes

5, and 6 exhibited reactivity to bovine immunoglobulins. Epitope 2 was the only one that did not

react to both the serum from the colostrums deprived calf and secondary antibody-enzyme

conjugate showing that this epitope is the only one responsible for inducing the specific immune

response when calves were either vaccinated with rPlpE or *M. haemolytica.*

Please replace paragraph [0059] with the following amended paragraph:

[0059] A closer examination of epitope 2 shows that this is part of the region identified as

having 8 imperfect repeats of hexapeptides (Pandher et. al., 1998). The 11 peptides (#13 through

23) identified here as epitope 2 comprise the last 4 residues of the 2nd repeat described by

Pandher et al., (1998) and the rest of the repeats i.e., repeats 3 through 8 with the exception of the

1st hexapeptide. A feature of these 11 peptides is the lack of uniformity in their binding capacity

as evidenced by the variation in their signal intensities. Peptides #15, 17, and 19 exhibit the

highest signal intensities followed by #s 21 and 23. The first 4 residues of the N-termini of these

peptides are ~~QNAPQ......~~ Q, N, A and P, with the exception of #21 in which the first glutamine

is replaced by glutamate. It is worthwhile noting that both glutamine and aspargine are positively

charged, with hydrophobicity index of - 0.91 and - 0.92, respectively. The remaining 6 peptides

in epitope 2 have proline at their N-termini instead of glutamine and this may account for their

relatively lower signal intensity in the peptide array. The relatively high signal intensities

exhibited by peptides 15, 17, and 19 may reflect the manner in which these epitopes are

presented to the immune system under natural condition on the surfaces of *M. haemolytica* cells

and the inherent immunogenic nature of these stretches of amino acids. The fact that epitope 2

contains significant number of prolines at defined intervals which are usually indicators of turns,

has an unusually high number of very basic residues such as glutamine, aspargine and glutamate

which are hydrophilic with high surface probability and 8 repeats are features that are usually

associated with regions of protein that are associated with being immunogenic. Moreover,

computer analysis of the deduced amino acid sequence of epitope 2 with algorithms such as

Parker's antigenicity, Kyte/Doolittle hydrophilicity, surface probability and Chou Fasman □°

structure indices show that the stretch of amino acids has a moderately high antigenicity, fairly

hydrophilic, contains fairly high number of amino acids with very high surface probability and is

characterized by series of turns associated with helices and sheets, respectively, all of which are

strong indicators of a region that is potentially highly immunogenic.